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Structure, Function and Clinical Detection of the Human Multidrug Transporter ABCG2, a Molecular Target in Anticancer Therapy

1. Introduction

The ATP-binding cassette (ABC) transporter family constitutes one of the largest families of membrane transporters present in all aspects of life. These proteins are involved in energy-dependent transport and play a critical role in normal physiology. A subset of these transporters governs the oral bioavailability of drugs, the penetration of drug substrates into sanctuary sites such as the central nervous system, the protection of stem cells in hematopoietic and other normal tissues, the excretion of substrates from liver and kidney, and the resistance to both antimicrobial and anticancer drug therapies. Emerging data suggest that ABC transporter expression in cancer stem cells may determine the presence of residual cancer cells following anticancer therapy.

A more recently identified transporter, ABCG2 (or BCRP), has been observed in several human cancer cell lines selected for drug resistance as well as in tumour samples of cancer patients. Studies of ABCG2 aiming at the molecular mechanisms of drug recognition and transport, and the development of strategies that circumvent ABCG2-mediated anticancer drug resistance are of critical importance. In the following list key areas of research under study in the Bates and Van Veen laboratories are highlighted, forming an excellent basis for a research project in this graduate program. The goal is to exploit ABCG2 as a molecular target for anticancer therapy.

2.1 Structure of ABCG2 - ABCG2 is a 655-amino acid, 72.1 kDa protein, which is a member of the G subfamily of human ABC transporters and thought to dimerize to form a functional transporter, although the dimer interface has not been resolved. Further, the domain organization of the G subfamily is reversed compared to most ABC transporters, such as P-glycoprotein (ABCB1). Recent observations suggest that the protein exists as a tetramer or higher order structure. The biological implications of these observations have not been explored.

2.2 Multidrug recognition - ABCG2 exhibits an extraordinarily broad drug specificity, which is very different from the stereochemical precision with which 'normal' cytosolic enzymes and membrane transporter for solutes recognize their substrates. The molecular basis of the broad specificity of ABCG2 is not understood at present, and is a challenge of current research in this area. Since ABCG2 is known to transport both amphiphilic cationic compounds and hydrophilic anionic compounds, this raises interesting questions about the amino acid residues responsible for drug-protein interactions. The Van Veen group has generated truncated forms of ABCG2, allowing scrutiny of drug binding in the absence of ATP hydrolysis and transport.

2.3 Role in sterol transport - Other Members of the ABCG subfamily have been implicated in the transport of sterols, and there is evidence that ABCG2 is involved in the transport of unconjugated sterols, sulfated conjugates of bile acids and steroids, and glucuronide conjugates of sterols. At present the sterol binding sites remain to be identified, and whether binding sites for sterols and drugs are dedicated, or whether these sites are overlapping. Could sterols be used to modulate drug transport by ABCG2?

2.4 Effect of single nucleotide polymorphisms on function - As ABCG2 is expressed in the placenta, liver, small intestine, colon, blood-brain barrier, and in stem cells, it is thought that ABCG2 has a physiological role in protection of tissues against xenobiotics, in addition to its role in cancer chemotherapy resistance. Interestingly, single nucleotide polymorphisms have been detected in the ABCG2 gene, but their role in altering the pharmacology of anticancer and other drug substrates has not been determined.

2.5 Clinical detection of ABCG2 - One important cause for the failure in the clinical development of P-glycoprotein transport inhibitors was the lack of a validated assay for detection. As inhibitors are developed, assays will be needed to determine which patients are most likely to benefit. Validated functional assays, imaging assays, immunoassays and RNA assays are needed for this purpose, and later to confirm inhibition of drug efflux in the clinical setting.

3. Host laboratories - The laboratories of Drs. Bates and Van Veen study multiple, but complementary aspects of ABCG2 - evaluation of structure and function (including dimerization and transport activity), identification of substrates and inhibitors, detection in cancers and/or cancer stem cells, and regulation of expression. Projects other than those above can be considered if they relate to the development of ABCG2 as a therapeutic target in cancer.

The Van Veen lab has experience in the overexpression of membrane proteins, their purification and functional reconstitution in artificial phospholipid bilayers. The laboratory is equipped to undertake molecular biological methods for modifying gene expression and for manipulating and analysing RNA, DNA and proteins including Northern, Southern and Western blotting, immunocytochemistry, transport studies with radiolabelled and with fluorescent tracers. Facilities are also available for visualising and quantifying intracellular distribution of fluorescent substrates, analysing intracellular pH and measuring ion transport activity using intracellular recordings.

The Bates laboratory has studied many aspects of the biology of drug resistance and has been involved in the translation of therapeutic strategies aimed at drug resistance reversal from the laboratory to the clinic. The laboratory has generated numerous drug resistant cell lines that allow study of expression and function of ABCG2 and has contributed to the extensive list of substrates and inhibitors currently available. Experience with expression vectors allows the study of particular amino acid residues that can provide a basic understanding of protein structure and function. The laboratory is committed to developing and implementing assays to detect ABC transporter expression and activity in clinical samples.

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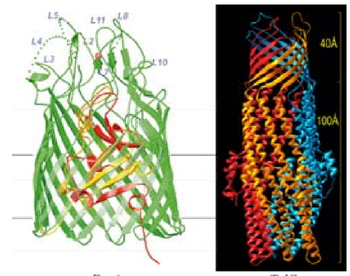

FepA TolC

Type V Secretion in Prokaryotes: Structure Determination of an Autotransporter

Many Gram-negative bacteria are pathogenic: Rickettsia, Bordetella, Neisseria, Shigella, Haemophilus, and other organisms all secrete toxic proteins to achieve their physiological goals. In order to transport virulence factors across the inner and outer membranes, five types of secretion pathways are utilized. Type V secretion is by far the simplest pathway. In this system, the outer membrane transport pore is contained within the precursor of the secreted protein itself, earning the nickname 'autotransporter' for proteins that secrete themselves. Although this secretion system contains a single protein, little is known about the molecular mechanism of transport or about the architecture of the transporter.

Sequence analysis of autotransporter genes suggests that they code for proteins divided into three domains. The N-terminal domain contains the virulent 'passenger' or 'cargo' domain which is secreted to the cell surface. The cargo domain may remain attached at the cell surface or it may be cleaved by a protease for release into the extracellular environment. The cargo domains of autotransporters vary in size, with many in excess of 100 kDa. The C-terminal domain is predicted to contain a 14-16 stranded transmembrane beta barrel, which inserts into the bacterial outer membrane and forms the transport pore. The structures of a 12- and a 22- stranded transmembrane beta barrel are shown below. The size of the predicted pore suggests that the cargo domain would be threaded through the beta barrel in a partially unfolded state. In between the N- and C-terminal domains lies a linker domain whose function is to allow the entire cargo domain to reach the cell surface while tethering the secreted cargo to the beta barrel.

The goal of this project is to determine the high resolution structure of an autotransporter protein by X-ray crystallography. The student selecting this project will first screen several autotranporters from various bacteria for high-level expression in E. coli. In addition to expressing the full-length proteins, different N-terminal cargo domains will be attached to the beta barrel transporter to increase the likelihood of crystallisation. The selected autotransporter will then be chromatographically purified and crystallised in the presence of detergent. Crystals will be analysed by X-ray diffraction techniques and the structure will be solved using isomorphous replacement or anomalous diffraction methods. Once the project is completed, the student will have gained experience in all aspects of membrane protein structural biology: from gene cloning to protein expression, purification, crystallisation, and structure determination by X-ray crystallography. Furthermore, the student will gain an understanding of the molecular basis for type V secretion, important for the virulence of numerous pathogens.

Further reading:
Autotransporters
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Inducible Inactivation of Gene Activity in Drosophila and Zebrafish

Model organisms provide an opportunity to study fundamental aspects of human biology and related diseases in simplified systems that are more readily manipulated. Two popular model systems with well-developed genetic tools are the fruit fly Drosophila melanogaster and the zebrafish Danio rerio. One common approach in both flies and fish is to randomly generate mutations in genes and then study the resulting effect of such mutations on the normal development or cell function. Generally these mutations eliminate or severely cripple the normal functioning of the gene. Many genes have more than one role in the developing organism and the typical "null" mutation will only reveal the earliest steps of development or cell biology that a given gene is involved in. This limitation can be addressed by the use of temperature-sensitive mutant alleles, which typically have normal gene function at a lower temperature and defective gene function at a higher temperature. However, such alleles exist only for a minority of all genes that have been mutated, not to mention the majority of genes for which no mutant alleles yet exist. When temperature-sensitive alleles do exist, they allow researchers to control the presence or absence of gene function by a simple temperature shift, and hence to investigate the role of the gene at any stage of development, both by analysis of the mutant phenotype, and the ability to recover large quantities of mutant material for biochemical analysis. Such alleles are therefore widely used, e.g. the shibirets alleles of Drosophila, which affect dynamin function, have been instrumental in investigation of membrane traffic and synapse biology, and FlyBase lists 78 citations for the shits1 allele alone (http://fly.ebi.ac.uk:7081/.bin/fbidq.html?FBal0015610). In zebrafish, it is very common for genes to have multiple roles in development, making phenotypic analysis very complex. It is also very labor intensive to isolate mutant alleles in zebrafish and, to date, no temperature sensitive alleles have been identified. While spatially or temporally controlled expression of stem-loop RNAi constructs now offers some possibilities for controlled induction of mutant phenotypes, it does not lead to the immediate and acute loss of protein that the degron approach does and that is invaluable for studies of the biochemistry and cell biology of mutants, it does not allow expression of functional protein from rescue constructs, and the need to make stem-loop constructs individually does not allow the random mutagenesis approach that would be possible with a degron-GFP trap.

However, more systematic approaches to generation of temperature sensitive alleles are now possible. Dohmen et al. (Science, 1994) identified a fragment of the budding yeast gene DHFR that when fused to any gene in yeast, conveyed temperature sensitivity to that gene product, by conferring temperature-sensitive accessibility of an N-terminal non-methionine residue that targeted the protein for degradation. This N-end rule for degradation of non-Met N-termini is essentially similar from yeast to mammals (Varshavsky, Genes Cells 1997). Hence, at 23°C the proteins were fully functional, but at 37°C, they were targeted for degradation.

Initial experiments to use this "degron" in Drosophila were promising, but not yet ideal (Nairz & Hafen, 1999; Rodesch et al., 2001). Temperature-sensitive gene activity was observed, but the highest temperatures at which flies can be cultured (about 30oC) do not entirely inactivate gene function. In addition, the GAL4 system used to express the degron fusions in flies is more active at higher temperatures, thus counteracting the efffect of increased protein degradation. To date, use of the degron has not been attempted in zebrafish, but the temperature range at which zebrafish can be cultured (from 24°C to 32°C) should make it feasible with an optimized degron.

The O'Kane and Burgess labs propose a joint project where systematic mutagenesis and screening of the DHFR degron is done to optimize the temperature profile of the degron for use in both flies and fish. This will be done by fusing randomly mutagenized versions of the DHFR degron to the yeast URA3 gene, then selecting for function of URA3 at the permissive temperature on -uracil plates and for loss of function of URA3 at the non-permissive temperature on 5-FOA plates. This simple screen should quickly identify a degron in the desired temperature range. Degron mutants with temperature activity profiles best for flies and fish will be used to generate conditional alleles in both organisms. Firstly, relevant degrons will be tested in both organisms, by fusing them to GFP expressed from a strong (but not heat-inducible) promoter, which will be a sensitive test for low protein levels. After this the student will make a choice of organism and genes to concentrate on. Degrons that perform best in the initial testing will be fused to products of genes being studied in both the O'Kane and Burgess labs (e.g. synaptic proteins in the O'Kane lab, zebrafish POU2 in the Burgess lab), and used for study of these proteins within each lab. A variant on this theme would be to use a "degron-GFP-trap" similar to the GFP-trap of Morin et al (2001), which could in principle produce functional temperature-sensitive protein fusions to any gene product with a cytoplasmic N-terminus, thus allowing large-scale and rapid generation of ts alleles of a large fraction of genes in the fly genome. Dr. O'Kane is a coinvestigator on a project that is making insertional mutant flies available to the Drosophila community (http://www.gen.cam.ac.uk/~flychip/p_elements/index.html), and this potentially offers a context in which such ts alleles could be generated on a larger scale and made available to the wider community.

This project provides an opportunity for a graduate student to learn techniques in three different model organisms - yeast, fruit flies, and zebrafish as well

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Defining the Genetic Influence of the Killer Immunoglobulin-Like Receptor Loci on Human Disease

Natural killer (NK) cells are an important component of the innate immune system and provide the first line of defense in the early stages of the immune response against viral infections and tumors, by production of cytokines and direct cytotoxicity of target cells. The killer immunoglobulin-like receptors (KIR) on NK cells regulate inhibition and activation of NK cell responses through recognition of their ligands, the HLA class I molecules, expressed on target cells. The genes encoding KIR and HLA are both highly variable across individuals and particular HLA class I products bind and trigger specific KIR molecules on the NK cell surface, raising the possibility of a synergistic relationship between KIR and HLA in NK cell mediated immunity.

The Carrington/Trowsdale laboratories, which have already collaborated informally, will combine their expertise in genetics and immune recognition to study interaction of HLA and KIR.

We propose to study whether beneficial and, conversely, detrimental combinations of genetic variants of the KIR and HLA loci might exist, influencing resistance to infectious diseases and to tumorigenesis. Disease cohorts, such as AIDS and psoriatic arthritis will be genotyped at the KIR and HLA loci, followed by statistical analyses that take into account multiple variables (multivariate regression analysis). Once relevant combinations of particular HLA class I molecules and their KIR partners have been identified genetically, interaction of the proteins they encode will be studied.

This approach represents a powerful tool for understanding disease pathogenesis and for development of therapeutic reagents against the disease. This is the first time such a powerful, rational approach to combinations of polymorphic defense genes has been possible. It will provide a model for similar approaches to other multigenic disorders.

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Mitotic functions of SUMO-1

SUMO-1 is a small ubiquitin-like protein that becomes covalently linked to other cellular proteins in a manner similar to ubiquitin. Rapid progress has been made in determining the components of this pathway, revealing that it is well conserved across species from yeast to mammals. Genetic evidence in yeast suggests that this modification is required for progression through mitosis, although the nature of this requirement is poorly understood. The Dasso laboratory has been studying the SUMO-1 pathway in vertebrates, and their studies indicate that mitosis is also dependent upon the SUMO-1 pathway in higher eukaryotes. They have also demonstrated that the mitotic localization of at least on critical target, RanGAP1, is dependent upon modification by SUMO-1. The Pines laboratory has developed sophisticated single-cell live imaging technology for examination of mammalian tissue culture cells in mitosis. They have used these technologies to extensively characterize the behavior and control of critical mitotic regulators during mitosis, particularly concentrating on the cyclin family of kinase accessory proteins. The project will employ live imaging techniques for characterization of mitotic defects after disruption of the SUMO-1 pathway. Initial experiments would examine how SUMO-1 regulates spindle formation, the transition from metaphase to anaphase and cytokinesis. Subsequent studies would utilize the expertise of both groups to characterize the regulation of the SUMO-1 pathway in mitosis and to determine the molecular mechanism by which it impinges on the mitotic regulatory machinery. These studies should provide important insights into the eukaryotic cell cycle, a fundamental and highly conserved mechanism whose misregulation leads to human diseases, such as cancer.

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Identification of Regulatory Networks in Culture Models of Cell Differentiation

The aim of this project is to identify regulatory networks in culture models of differentiation. The student will be able to compare the differentiation program in two different cell types, using a mammary epithelial cell culture model which can be induced to differentiate, form 3-D cultures and express milk proteins (in the Cambridge laboratory) and U937 cells, that can be induced to differentiate with retinoic acid (in the NIH laboratory). Changes in the pattern of expressed genes as these cells differentiate and pass through differentiation checkpoints will be determined using microarray analysis coupled with sophisticated bioinformatics packages. Genes critical for the regulatory networks will be determined by comparing the cell systems and using RNA interference knockdown technology to specifically abrogate function at specific times in the differentiation program. It may also be possible to use an in vivo approach with transgenic mice. The results may have implications for our understanding of the mechanisms of cell differentiation and development of novel approaches for treatment of cancer. The student will join active and enthusiastic research groups with interests in signaling and apoptosis in mammary gland (Cambridge), and differentiation and apoptosis of cancer cells including the role of telomerase (NCI, NIH).

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Proteoglycans and Nervous System Regeneration

Nerve fibre growth and regeneration in the nervous system

When nerve fibres in the peripheral nervous system (i.e. those innervating skin and muscle) are cut or damaged, they are able to regenerate. In contrast, nerve fibres in the central nervous system (CNS) (i.e. brain and spinal cord) will not. This failure to regenerate is largely due to CNS glial cells which produce molecules that inhibit nerve fiber growth. We have shown that astrocytes, the most plentiful of the CNS glial cells, secrete inhibitory sulfated proteoglycan molecules into the extra cellular matrix which surrounds the cells. Inhibition can be attributed to both the protein core and the sulfated sugar side chains. We are presently working to identify which proteoglycan is responsible, and to develop clinically usable ways to prevent it blocking nerve fibre growth.

An alternative approach to inducing nerve fiber regeneration in the brain and spinal cord is to alter the signaling within growth cones so that neurons no longer stop at proteoglycans. We have already shown that altering the Rho GTPase signal transduction pathway can allow neurons to grow across inhibitory boundaries. We are presently working on extending these findings to other signals in the growth cone and also to test this strategy in animal models of regeneration.

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The Structure and Function of Vg1RBP

This project seeks to combine the strengths of the Standart and Hall labs in structural and functional studies of RNA-binding proteins to study Vg1RBP. Vg1RBP is a member of the highly-conserved ZBP (zipcode-binding protein) family of RNA-binding proteins. ZBP proteins control RNA localization, translation and turnover by binding to cis-acting sequence elements in the 3´ untranslated regions of target mRNAs. ZBP proteins are classified as oncofetal proteins as they are normally expressed during early embryogenesis but may be overexpressed in cancer (reviewed in [1,2]). Xenopus Vg1RBP functions in development by transporting Vg1 and VegT mRNAs to the vegetal pole during oogenesis.

Vg1RBP comprises 2 N-terminal RNA recognition motifs (RRMs) and two tandem pairs of KH-domain didomains. The KH didomains mediate RNA-binding and oligomerisation, while the 2 RRMs seem dispensable for interaction with known RNA targets. Both didomains, KH12 and KH34, appear to be important for RNA interaction. The second KH didomain, KH34, is a dimerization module, which stabilizes cooperative RNA binding by two protein molecules [3,4]. Structures of single KH domains, with and without RNA, have been determined. KH domains in general appear to recognize tetranucleotide motifs. Thus, biological RNA targets of KH domains may use either RNA secondary structure or repeated sequence elements to achieve high affinity and specificity of binding. Structural information regarding RNA binding by multiple KH domains, in any protein, is lacking.

The aims of this project are to i) express and purify Vg1RBP KH12, KH34, and KH1-4 domains, ii) determine the minimal RNA element specifically bound by these domains [3,5,6], and iii) determine the structures of the KH domain fragments alone and/or in complex with RNA. If time permits, the student may also investigate whether phosphorylation of Vg1RBP has an effect on RNA binding, as was observed for chicken ZBP. These studies will provide insight into the use of KH domain modules for RNA recognition and protein-protein interaction. The student will have the opportunity for training in molecular biology, biochemistry, and structural biology.

The student will begin at Cambridge and optimize expression of the KH domains in E. coli, undertake RNA-binding studies, and perform X-ray crystallisation trials, if time allows. His-tagged constructs, capable of yielding large amounts of soluble proteins, have been created [3] and will constitute the starting material. The student will later move to the NIH (North Carolina) to continue or begin crystallisation trials of the proteins and protein:RNA complexes. When crystals are obtained, the student will collect X-ray data and determine the structures. Follow-up biochemical studies may be performed in either laboratory.

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T1DM Immunology and Immunotherapy Research

Type 1 diabetes mellitus (T1DM) is a devastating illness affecting millions of people worldwide, including an estimated 1 million Americans, and 100,000 citizens in the United Kingdom. T1DM is thought to result from an autoimmune destruction of the insulin producing beta cells in the pancreas (islets). While it is well established that individuals with T1DM can, by keeping their blood sugars normal, prevent the blindness, kidney failure, nerve damage, and premature mortality that otherwise accompanies the disease, it is also recognized that maintaining normal blood sugars by delivery of exogenous insulin is extraordinarily difficult.

This collaborative project has two major themes. One, considerable animal model and clinical data suggest that the pancreatic islet insulin producing beta cells may have significant regenerative capacity. NIH and Cambridge investigators will explore the biology underlying this apparent regenerative capacity in an effort to promote beta cell regeneration for therapeutic benefit. Two, several features underlying the autoimmune beta cell destruction, e.g. the slow pace of the beta cell killing that takes months to years, and the patchy nature of the destruction whereby one islet will be destroyed while a neighbor may appear unscathed, remain enigmatic. Animal models developed at the NIH and at Cambridge University will allow program participants to address immunological questions underlying disease pathogenesis. The project will take advantage of these experimental small animal models and clinical material available at the NIH and in Cambridge. Integration of these data will facilitate the development of novel therapeutic agents with the potential for inhibiting immune mediated islet destruction and/or promoting islet cell regeneration. Participants in the Cambridge - NIH Program for T1DM immunology and immunotherapy research will develop expertise in immunology, the genetics of T1DM susceptibility, and will have considerable exposure to beta cell biology.

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Cancer Predisposition and the Functions of the BRCA2 Tumor Suppressor

Inherited mutations in the breast cancer susceptibility gene BRCA2 predispose to cancers affecting specific tissues such as the breast, ovaries and prostate, with a high penetrance estimated at 30-70% by age 70 years. The large nuclear protein encoded by BRCA2 bears little resemblance to molecules of known function. There is increasing evidence that BRCA2 plays essential roles in the biological response of mammalian cells to DNA damage, through functions in pathways for DNA recombination that preserve chromosome structure, and in the control of cell cycle during mitosis. However, these functions appear to be fundamental to all cells, raising the important but unresolved question as to why BRCA2 mutations predispose humans to cancers confined in specific tissues such as the breast (reviewed in 1).

To address this important question, the collaborative project will bring together the complementary expertise available in the labs of Dr Lothar Hennighausen (Chief, Laboratory of Genetics and Physiology, NIDDK, NIH) and Professor Ashok Venkitaraman (University of Cambridge and the Medical Research Council Cancer Cell Unit). The Hennighausen lab (2-4) has pioneered the use of genetic systems for the analysis of pathways in the differentiation and function of epithelial cells in the breast, using a broad range of approaches including targeted gene disruption in the mouse germline, and analyses of gene expression using DNA microarrays. The Venkitaraman lab (5-7) has made important contributions to current understanding of the biological functions of BRCA2, using genetic, cell biological and biochemical techniques for the analysis of chromosome structure, DNA repair, replication and the cell cycle.

The collaborative project will afford candidates with training in a broad range of modern techniques in molecular and cell biology, applied to a problem of central importance in cancer biology, with relevance to the treatment of cancer. The collaborating labs at the NIH and in Cambridge encourage interactive, focused, hypothesis-driven science. They have access to state-of the art laboratory facilities for the proposed work. Further information concerning the project and the collaborating labs may be obtained by writing to Professor Venkitaraman (arv22@cam.ac.uk).

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Project Title

The ear is a biological system of special interest for physicists for various reasons. For example, its detection threshold is about the energy level of thermal agitation and yet it has a wide dynamic range. It not only converts mechanical signal into electrical one but functions also as a frequency analyzer. It operates rather high frequencies based on mechanical resonance in a viscous fluid. It has compressive nonlinearity, which is interesting as a dynamic system, to achieve the wide dynamic range.

We try to study the physical and biophysical principles of hearing by combining experimental (primarily at NIH) and theoretical approaches (primarily at Cambridge). One key area of our interest is in mechanoelectrical transduction that includes ``cochlear amplifier,'' active cellular processes with which other forms of energy is pumped in to mechanical vibration and thereby the ear achieves its low hearing threshold as well as sharp frequency discrimination. We are interested in studying such processes that operate at the rather high auditory frequencies from the view points of molecular machine as well as that of a dynamic system.

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Non-integrating Human Immunodeficiency Virus

Lentiviral vectors are considered useful for gene therapy because such vectors can successfully transduce genes into non-proliferating cells. During transduction, lentiviruses integrate obligatorily into host cell chromosomes. The adverse risks from such integrations in vivo are poorly understood, and have been thought by some to be largely theoretical. However, a recent report of the development of leukemia in a patient secondary to gene therapy employing a retroviral vector illustrates the real potential for insertional mutagenesis in vivo.

One solution which addresses this risk is to create lentiviral vectors which can be stably maintained but cannot integrate into host chromosomes. Toward this goal, we have embarked on designing an HIV-1 based vector which accomplishes both objectives. Our approach is to point mutate the HIV-1 genome in its integrase gene thereby creating a virus which is integration defective. To ensure its maintenance as a stable episome in mammalian cells, we will additionally place the plasmid origin of replication (Ori P) from the EB virus into this genome. In the presence of the EBV protein, EBNA-1, Ori P containing DNA is stably maintained as non-integrating episomal DNA. We envision providing EBNA-1 in trans from another expression vector or alternatively we would insert an EBNA-expressing reading frame into a portion of HIV-1 which is not absolutely required for viral replication (e.g. nef). The resulting non-integrating lentiviral vector should be safer than current counterparts.

This project will be a collaborative study between the laboratories of Dr. Andrew Lever at Cambridge University and Dr. Kuan-Teh Jeang at the NIH.

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Project Title

The two supervisors have a common interest in the area of retroviral research and particularly the human pathogenic retroviruses HTLV-1 and HIV. Dr Jeang has worked extensively on transcriptional control in these viruses and more recently identified a critical host chaperone protein DDX3 responsible for nuclear export of HIV-1 transcripts. Professor Lever has worked extensively on RNA packaging and recently observed that the packaging signal in the viral RNA of HIV influences the subcellular targeting of both RNA and the site of protein capture. A variety of PhD projects is available involving the combined expertise of the two laboratories. It is likely that the subject matter will focus on cellular transport mechanisms involved in viral RNA export and viral assembly however the exact details will be decided in discsussion between the supervisors and the student.

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The Functions of Cyclin-Dependent Kinase 2 (Cdk2)

Cyclin-dependent kinases (Cdks) are thought to promote the transitions between the different phases of the cell cycle. The cyclin-dependent kinase 2 (Cdk2) has been suggested to promote entry in to S-phase, initiation and maintenance of DNA replication, entry into the G2 phase, and maybe even entry into mitosis. In order to fulfill these functions, Cdk2 needs to be activated by binding to cyclin E and cyclin A. We have generated Cdk2-/- knockout mice and found that they are viable. This is very surprising, among other reasons because cyclin A2-/- mice are early embryonic lethal. To study the absence of Cdk2 in more details, we wish to investigate the behaviour of the cyclin E- and cyclin A-Cdk complexes in Cdk2-/- mice, and in cells derived from these mice. We will employ biochemical methods and microscopy to study which cyclin-Cdk complexes are present at specific points in the cell cycle, and elucidate their activation, and their inactivation by cyclin destruction, by phosphorylation of the Cdk and by binding to CDK-inhibitors. In particular we will test whether Cdk1 is able to perform the functions of Cdk2 at S phase and, if so, whether this is through cyclin A or a novel cyclin E-Cdk1 complex. We will also study the localisation of the cyclins in living cells because we have found that cyclins A and B1 need to bind their Cdk to be transported into the nucleus. We will approach these questions by a biochemical analysis of the cyclin-Cdk complexes and by time lapse microscopy using cyclin-GFP chimaeras to analyse localisation and cyclin destruction.

We envisage that the time-lapse microscopy studies will be performed in Cambridge and the biochemistry at the National Cancer Institute, Frederick.

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Molecular and Cellular Mechanisms of Immune Regulation in Diabetes and Associates Immune Disorders

Type 1 diabetes and Sjogren's syndrome are autoimmune diseases that occasionally occur together in humans. The NOD mouse provides an excellent animal model of human Type 1 diabetes and interestingly also develops Sjogrens syndrome as characterised by the presence of a mononuclear cell infiltration of the lacrimal and salivary glands and the development of both anti-salivary duct antibodies and anti-nuclear antibodies. In this mouse strain the anti-nuclear autoantibodies are directed against components of the splicesome. There is some suggestion that there are some defects in apoptosis in the NOD mouse which might contribute to defective central and peripheral tolerance. Autoreactive T cells, once primed, might escape the normal processes in place for maintenance of peripheral tolerance. Therefore dendritic cell presentation of nuclear antigens to autoreactive T cells might go unchecked and be able to provide help for autoreactive B cells resulting in the generation of anti-nuclear autoantibodies. As NOD mice also appear to have defective FcgRIIb expression the B cell autoantibody response would not be subject to feedback regulation. We will study molecular regulation of B and T lymphocyte activation and programmed cell death in cells from patients with these disorders to uncover the pathogenetic basis of immune dysfunction.

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Diversification of Immunoglobulin Genes

The laboratories of Drs., Michael S. Neuberger, Medical Research Council Laboratory of Molecular Biology (Hills Road, Cambridge CB2 2QH, UK) and Dr. Rose G. Mage, Laboratory of Immunology, NIAID, NIH, Bethesda MD 20892 1892 have shared interests in the development and diversification of the B-cell antibody repertoire. In specific sites such as developing gut associated lymphoid tissues of chickens and rabbits, an early pre-immune repertoire of B-lymphocytes develops. The rearranged genes that encode antibody heavy and light chains undergo diversification of their DNA sequences by mechanisms of gene conversion and somatic hypermutation. During antigen-specific immune responses, these mechanisms lead to further diversification of antibody sequences in the germinal centers of secondary lymphoid tissues such as lymph nodes, spleens and Peyer's patches. Among the current goals of both laboratories are developments of further understanding of the mechanisms that lead to the diversification of these gene sequences.

Further information about Dr. Mage's research and publications can be found at:
<http://gpp.nih.gov/faculty/viewbook/Mage_Rose.html> and <http://www.niaid.nih.gov/dir/labs/li/mage.htm>

Two recent references to Dr. Neuberger's work are:

Sale, J. E., D. M. Calandrini, M. Takata, S. Takeda, and M. S. Neuberger. 2001. Ablation of XRCC2/3 transforms immunoglobulin V gene conversion into somatic hypermutation. Nature 412:921.

Sale, J.E. And M.S. Neuberger. 1998. TdT-accessible breaks are scattered over the immunoglobulin V domain in a constitutively hypermutating B cell line. Immunity 9:859.

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Functional Immunogenetics of Cell Surface Receptors

This scientific partnership is established between the laboratories of John Trowsdale, Head of the Immunology Division of the Department of Pathology of the University of Cambridge, and David H. Margulies, Chief of the Molecular Biology Section of the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases (NIAID), NIH. Dr. Trowsdale's interests have been in the immunogenetics of the genes of the human major histocompatibility complex (MHC) and the genes that encode natural killer (NK) cell receptors. His laboratory has been instrumental in evaluating the genomic organization of the human MHC and NK complexes, and has also examined the function of a number of MHC-linked genes in antigen presentation. Recent work has defined a novel set of MHC class I like genes that are expressed in tumor cells and that may play a role in recognition by NK cell activation receptors. Dr. Margulies' laboratory has been interested in the molecular interactions of T cell receptors (TCR) and NK receptors with their MHC ligands, and has expressed examples of each of these in recombinant systems to allow their biophysical and structural analysis. This work has resulted in the measurement of affinities of MHC-I molecules for TCR and NK receptor ligands and recently has allowed the definition, both structurally and functionally, of the precise site of interaction of an inhibitory murine NK receptor with its MHC-I ligand. The collaborative project between the Cambridge and NIH laboratories will take advantage of the Trowsdale laboratory's recent identification of unique human genes that encode MHC-like potential NK receptor ligands and the Margulies laboratory's expertise in engineering of recombinant proteins and quantitative analysis of their molecular interactions. The co-sponsored student will also have the unique opportunity of participating in a project that bridges human genomics and proteomics in a way that should permit translating the knowledge and identification of new genes into defined biological function. The molecular interactions explored are expected to elucidate mechanisms that contribute to normal immune responses as well as to autoimmune conditions. The unique environments of the Department of Pathology at the University of Cambridge and the Laboratory of Immunology of the NIAID, NIH, will foster an understanding not only of contemporary immunology, but also of molecular immunogenetics and structural biology.

Descriptions of the laboratories' programs are found at:
http://www-immuno.path.cam.ac.uk/~immuno/jt.html
http://www.niaid.nih.gov/dir/labs/li/margulies.htm
http://myprofile.cos.com/marg87779

Several relevant recent publications include:

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2. Trowsdale J. 2001. Genetic and functional relationships between MHC and NK receptor genes. Immunity 15: 363-74.
3. Allen RL, Raine T, Haude A, Trowsdale J, Wilson MJ. 2001. Leukocyte receptor complex-encoded immunomodulatory receptors show differing specificity for alternative HLA-B27 structures. J Immunol 167: 5543-7.
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5. Wang J, Whitman MC, Natarajan K, Tormo J, Mariuzza RA, Margulies DH. 2002. Binding of the natural killer cell inhibitory receptor Ly49A to its major histocompatibility complex class I ligand. Crucial contacts include both H-2Dd AND beta 2-microglobulin. J Biol Chem 277: 1433-42
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Structure and Function of Natural Killer Cell Receptors: Multiple Molecular Solutions to Self, Nonself Discrimination. Annu. Rev Immunol.20, in press.

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A Family Genetic Study of Asperger Syndrome (AS)

One model of AS characterises the cognitive profile in terms of an empathising deficit in the presence of intact or superior systemising. Systemising is the ability to analyse or construct a system (of any kind).
Empathising is the ability to recognize another's mental states and feel an appropriate emotion in relation to their emotion. The proposed research will test if this cognitive profile is found in the 1st degree family relatives of those with AS, as well as in the patients themselves.

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DNA double-strand breaks (DSBs) are generated deliberately by organisms in order to facilitate biological processes such as mating type switching in yeast and generation of immunoglobulin diversity in vertebrates. These DSBs must be effectively sensed and processed by the cell in order to successfully carry out these functions. In contrast, unintentional DSBs in DNA can be caused by exogenous or endogenous sources, such as ionizing radiation, exposure to radiomimetic drugs, and oxidative damage. If left unrepaired, DSBs can result in cell death or genomic instability. A growing body of evidence indicates that in multicellular eukaryotes, this is a key event in the development of cancer. Therefore, the ability of a cell to appropriately detect and repair double-strand breaks in DNA is of vital importance on both a cellular and organismal level.

Gross chromosomal rearrangement (GCR) is one of genomic instabilities found in many cancers. DSBs presumably generated during DNA replication are major source of GCR formation. To understand GCR formation how it is suppressed and how it is enhanced, an assay that can detect GCR formation in yeast Saccharomyces cerevisiae was developed. Seven different GCR suppression pathways and five GCR promoting pathways were identified.

In eukaryotic cells, nuclear DNA is compacted by association with histone proteins to form chromatin. The basic unit of chromatin is the nucleosome, which is made up of 146 bp of DNA wrapped around two of each of four core histones; H2A, H2B, H3 and H4. DNA is further compacted by the organisation of nucleosomes into higher order structures. This process is mediated, at least in part, by interaction with linker histones.

Compaction of DNA in this manner appears to be inhibitory to many DNA-dependent metabolic processes. Moreover, numerous studies have demonstrated the fundamental importance, both in vivo and in vitro, of regulation of chromatin structure in mediating processes such as transcription and replication. The abnormal regulation of chromatin structure could generate un-desirable DSBs, which could be a source of GCR formation. Dr. Downs and Dr. Myung laboratories want to tackle the following questions with a motivated graduate student: "How is chromatin structure normally built; what proteins and pathways? Could abnormal regulation of chromatin structure promote GCR formation? Then, how and what pathways participate in GCR formation? Lastly, could it be conserved in the mammalian system?"

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Molecular mechanisms of DNA damage detection and repair

Chromosomal breaks are potent inducers of chromosomal aberrations and therefore are among the most dangerous threats to the integrity of the genome. Yet, we are continually faced with these potentially mutagenic DNA lesions since they arise spontaneously by reactive byproducts of oxygen metabolism, during DNA replication, and exposure to genotoxic agents. Double strand breaks also serve to initiate a number of recombination events such as meiotic recombination in germ cells and antigen receptor rearrangements in lymphocytes. Although much progress has been made in identifying some of the cellular constituents of the DNA repair pathways that protect the integrity of the genome, the mechanisms by which cells' monitor and repair chromosomal breaks remains largely unclear.

In this project, it is proposed to use molecular, cytological and biochemical approaches in both Saccharomyces cerevisiae and mouse systems to address the following issues: identify proteins that are the primary sensors of chromosomal breaks; determine how the recognition of DNA damage by such damage sensor leads to the accumulation and activation of DNA repair proteins on double strand breaks; and study the downstream signal transduction cascades that are triggered by DNA damage. The identification of important components in the DNA damage response pathway using yeast genetics will allow the development of new mouse models with defects in DNA damage signaling and repair.

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Project Title

This project focuses on the functional genomics and proteomics of spermatogenesis; an extremely well conserved biological process with many similarities between flies and mammals. Recent microarray work at the NIH (Dr. Brian Oliver) and Cambridge (Dr. Stephen Russell) has shown that in both humans and in Drosophila there are striking differences in gene expression between the testis and other tissues, but we know nothing about the function of the majority of these thousands of testis- biased or specific genes. The goal of this project is to identify evolutionarily conserved genes, which are likely to have important roles in spermatogenesis, and determine the function of the encoded proteins. Some of these genes will be important for the elaboration of the mature sperm cell from its post-meiotic precursor the spermatocyte. Others will be involved in the production of sperm cell progenitors from the germ cell precursors, providing an extremely tractable system for studying stem cell biology and the regulation of the cellular programs that switch cells from a proliferative self-renewing stem cell fate to a differentiation program. Many will provide insight into the genetic basis of idiopathic male sterility -- a significant problem for human couples. Our laboratories are taking genomics approaches to exploring the transcriptome of the Drosophila and human testis, using DNA microarrays (NIH and Cambridge) directed sequencing of Drosophila testis EST libraries, and comparative genomics (NIH) to discover the complement of genes expressed in the testis. We now have a substantial body of data that will allow us to discover sets of genes coordinately regulated during Drosophila spermatogenesis that have direct orthologues in mammalian systems. We propose selecting a set of conserved genes expressed in the testis of both humans and Drosophila and determining their role in Drosophila spermatogenesis. More specifically, genes with no known mutant phenotype in Drosophila will be targeted using the custom deletions being generated in Cambridge or other off-the-shelf collections of Drosophila mutations. Mutant phenotypes will be characterized morphologically and by global gene expression analysis. We will target the proteins encoded by some of this set of genes for a proteomics analysis, generating tagged fusion proteins that will then be used in immunopurification and mass spectrometry strategies to identify the components of protein complexes. We have begun a preliminary exploration of the testis proteome in the Cambridge Centre for Proteomics. We believe that the combination of the sophisticated genetics techniques available in the fly, coupled with modern approaches to genomics and proteomics will uncover conserved biological processes necessary for spermatogenesis in both flies and mammals.

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Interleukin-4, Inhibitory Receptors, and the Early Events in T Dependent B Cell Activation

Interleukin-4 (IL-4) is a pleiotropic cytokine produced by T cells which is particularly important in the B cell immune response. It is necessary for isotype switching, controls expression of a number of cell surface proteins, prevents B cell apoptosis, and acts as a co-mitogen (Nelms et al. Ann Rev Immunol 1999;17:701-738). We have recently shown that it releases the B cell from the effects of inhibitory receptors (IRs) such as CD22 and FcgRII, and reduces the expression of a number of them (Rudge et al. J Exp Med 2002; in press). This raises the intriguing possibility that the effect of IL-4 on B cell proliferation might be by removal of tonic inhibition rather than by direct activation. The physiological significance of these findings, and the precise biochemical mechanisms underlying them, are not known.

The student will use a unique mouse model to identify the early T-B interactions in which IL-4 is involved, and then use these findings to inform experiments examining control of B cell inhibition during these early interactions. A mouse expressing a chimeric GFP/IL-4 gene (Hu-Li et al. Immunity 2001;14:1-11) will be immunised with TH1 and TH2 promoting antigens, and draining lymph nodes etc. examined by confocal microscopy. Sites of initial T-B interaction involving IL-4 will be defined, and subsequent studies will examine the expression of IRs on the interacting B cells. Parallel studies will assess the signalling pathways subserving the IL-4 induced reduction in IR function, using reagents generated in Dr Paul's laboratory. A "back-up" project, if needed, will examine the mechanism by which IL-4/Stat-6 reduces IR expression on B cells. It is envisaged that the student would commence working at the NIH where initial experiments on the chimeric GFP/IL-4 mice would be performed, before moving to Cambridge.

This project will use a novel mouse model generated in Dr Paul's laboratory to examine the involvement of IL-4 in the early events in T dependent B cell activation. In particular it will investigate the significance and mechanism of action of the recent observation by the Smith laboratory that IL-4 may promote the B cell immune response by removing inhibition, rather than purely by activation.

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Neurocognitive Function Investigations In Adolescents At High Risk And Suffering From Early Onset Affective Disorder

Endocrine correlates of juvenile major depression

There are now a number of studies demonstrating a critical link between cortisol and DHEA secretory patterns and the onset, course and outcome of first episode affective disorder during the school age years. Evening cortisol hypersecretion and a higher cortisol/DHEA ratio during the first episode of juvenile depression is associated with a chronic depressive disorder, worsening psychosocial course and a recurrent outcome [1-3]. By contrast morning cortisol hypersecretion and the morning cortisol/DHEA ratio predicts onset and subsequent persistence (>2 years) of disorder in well adolescents at high risk for affective disorder [4-6]. Replication of these psychoendocrine findings is clearly an important next step.In addition the precise neuropsychological impairments and neural systems involvement that may occur as a result of these endocrine changes have yet to be conclusively established in either high risk subjects or those already ill.

It is well established that cortisol hypersecretion in MDD may impair affective-cognitive processes through deleterious effects on the structure and functions of the hippocampus [7] [8]}. Active neurogenesis, which occurs in the dentate gyrus well into adult life in rats, monkeys and humans, may be negatively modulated in part by glucocorticoids [9] [10]. Studies in human volunteers have demonstrated reversible declarative memory impairments following physiological doses of oral hydrocortisone [11] [12]. In early-onset major depression brain changes have been reported in the hippocampus, amygdala, caudate nucleus, putamen, and frontal cortex, structures (the limbic-cortical-striatal-pallidal-thalamic tract -LSCPT) [13-15] [16]. These neural system findings suggest that the pattern of psychological deficits may vary dependent on the extent of the neural involvement. They may also be more widespread than hitherto suggested. For example emotion regulation, attention behavioural inhibition , decision making and episodic event memories may show impairments at different stages in the evolving nature course and outcome of affective disorders. Whether such changes already exist in subjects at high risk for affective disorder is not known.

The current research programme in Developmental Psychiatry and Clinical Cognitive Neuroscience in Cambridge is involved with mapping the relations between cortisol and DHEA, different sub types of affective disorder and their intermediate psychologies, including emotion recognition and response, episodic event memory, biases in information processing, attention and executive functions. A new doctoral student would join a multi-disciplinary group in Cambridge (PI, Dr. Ian Goodyer ig104@cus.cam.ac.uk) and the Affective Disorders Research Programme at NIMH (Director, Dr. Dennis Charney, charneyd@nih.gov) to conduct further investigations into the psychoendocrine characteristics of affective disorders in children and adolescents. These may include studies of affective-cognitive regulation systems and their chemical and neural systems in adolescents at high risk for psychopathology. Particular emphasis will be placed on integrating findings in neuroendocrinology with those in cognitive neuroscience. Experience and training will be selectively available in endocrine measurement, psychological assessment, patient interview techniques and functional neuroimaging. Choice would depend on the precise nature of the research process.

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T Cell Anergy in Diabetic Insulitis

Diabetes is a prolonged disease process in which the immune system attacks and destroys the Insulin-producing beta cells of the pancreas. During this process a stage referred to as insulitis is observed in animal models in which the T cells have entered the pancreas but do not destroy the beta cells. Current work is focussed on trying to understand the regulatory mechanisms that operate at this time point, with the eventual hope that clinically one would be able to reinforce them to prevent the onset of the destructive phase of the disease. Dr Cooke's laboratory has developed several models in the NOD mouse for studying this immunoregulatory state. Dr Schwartz's laboratory has discovered and characterized the tolerance mechanism known as T cell clonal anergy. The joint project would attempt to determine whether anergy plays any role in the unresponsiveness of the T cells in the insulitis phase. Experiments will utilize T cells expressing green fluorescent protein to isolate and follow the fate of diabetogenic clones in the midst of other immunoregulatory T cells.

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Molecular Mechanisms of Cell Motility

Myosins were first described as the force generator in muscle contraction. It is now known that myosins are a large superfamily of actin-dependent molecular motors which participate in a diverse series of chores within cells including cytokinesis, phagocytosis, endocytosis, filopodial formation, maintenance of cell adhesion, stabilization of cellular structural integrity and movements of cellular cargo such as Golgi membrane, secretory vesicles, chromosomes and mRNA. The human genome contains about 40 myosin genes that can be catagorized into eleven different classes. Our two laboratories are interested in how these myosins intereact with actin on a molecular level, which tasks they perform within cells and organisms, what proteins they interact with in cells that allow them to be targeted to specific locations and how the function of a given myosin within a cell is regulated. To answer these questions we use a variety of biochemical, biophysical, cell biological and physiological techniques including single molecule mechanical assays using optical tweezers, production of transgenic animals (both mouse and Drosophila), GFP-tagged protein imaging in living cells and proteomic approaches. The Scholar will investigate the role of a novel myosin in cells, possibly combined with biochemical studies of the in vitro function of the myosin. State-of-the-art techniques will be used to understand what the myosin does within cells and how it accomplishes this feat.

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Project Title

A major focus in the NIH laboratory is related to understanding aging and regenerative senescence in mammary epithelium. This phenotype is considered to result from the loss of mammary epithelial stem cells and their self-renewing capacity. Our laboratory recently published a "proof of principle" that early growth senescence in mammary epithelium resulted in a significant protective effect to mammary cancer risk. P53 plays an essential role in stem cell function and maintenance. The loss of p53 in mouse mammary epithelium results in the indefinite survival of epithelial growth potential in serial transplants indicating that p53 function may be central in stem cell ageing.

The aim of this project is to identify and characterize key features of stem cells of the mammary gland and to establish stem cell cultures. The experimental approach will encompass a variety of techniques utilizing complementary expertise in the host laboratories including stem cell transplantation (in the NIH laboratory) and conditional gene deletion (in the Cambridge laboratory). We have already generated a number of genetically modified mouse strains, including LIF and IKKb deficient mice, which exhibit abnormal ductal branching and alveolar budding. Cells from these strains will be transplanted into cleared fat pads and their pluripotentiality and ability to self-renew determined. In addition, we will take a candidate gene approach and use RNAi knockdown technology to delete components of the Notch signaling pathway since this has been shown to be essential for stem cell maintenance in cells of neural origin.

This project will provide the student with training in a wide range of cell and molecular techniques including fluorescence microscopy and stem cell transplantation. The host laboratories will provide a stimulating environment and the opportunity to interact closely with other graduate students and postdoctoral scientists (10 in the Cambridge laboratory).

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Structural and Functional Analysis of Molecular Machines

It is now widely recognised that many of the most important biological processes, from gene transcription to signal induction to metabolism, are driven by the synergism of different protein molecules in complex assemblies and the specificity of the underlying protein-protein recognition that dictates assembly. Some of the most fascinating intracellular reactions are catalysed by multienzyme systems, capable of bringing about multistep reactions in elegantly ordered fashions that enhance efficiency and protect against side-reactions.

The goal of this international collaboration is to establish the three-dimensional (3D) structures of a particularly important family of giant multienzyme systems, the 2-oxo acid dehydrogenase complexes. This is a multi-disciplinary research project that takes advantage of the strong infrastructure that is in place for molecular biology, enzymology and protein engineering in Cambridge, and for structural biology, cryo electron microscopy and electron tomography at NIH. We expect that this work will lead to the detailed analysis of a complex multimolecular catalytic machine, throwing new light on the catalysis of multi-step reactions. There will be important lessons to be learnt about protein design and re-design and self-assembly. The structural analyses may have implications for applied research as well, for example in the design of novel molecular scaffolds displaying exogenous protein antigens could enable us to explore the ways in which an immune response can be evoked, with a high potential for exploitation in the development of new immunological tools, not least new approaches to vaccine design and T-cell therapy.

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Project Title

Human addiction is caused equally by gene variants and environmental features. Much of the genetic vulnerability to addictions is likely to influence its memory-like aspects. Interested students can help to identify human allelic variants that predispose to addiction, explore how these gene variants alter addiction's memory-like and rewarding aspects and find out which addiction-associated variants exert selective influences on which mnemonic processes in vitro, in mouse models and in humans.

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Project Title

A major focus is the study of cognitive function in relation to genetic polymorphisms, and with applicability to human neuropsychiatric disorders including schizophrenia, Parkinson's and Alzheimer's diseases. The Cambridge group has expertise in innovative human cognitive neuropsychology and functional neuromaging (functional magnetic resonance imaging, also employing PET to measure regional cerebral blood flow, and shortly, studies of dopamine ligand displacement). This group devised and applied the CANTAB computerised neuropsychological test battery and validated it on a wide variety of neurological and neuropsychiatric disorders, including patients with focal cerebral lesions. One major aim is now to investigate genes of interest for cognition as measured using these tests on a large number of healthy volunteers or on carefully evaluated clinical samples. The NIMH group under Dr Weinberger also has major competence in cognitive neuropsychology (Dr T. Goldberg) and neuroimaging in several modalities, as applied especially to schizophrenia. This group also has access to an excellent genotyping facility and has published several major papers in the last 3 years on genes of interest to neuropsychiatry, notably the COMT polymorphism and a recent study of the BDNF gene and its relevance to memory-which make them leaders in this fast-expanding field. The projects we wish to propose therefore involve a combination of neuropsychology and neuroimaging, probably to be conducted in Cambridge, in conjunction with the collection of blood samples for analysis by the NIMH group to test specific hypotheses which may be extended to patient groups. Therefore, students will acquire a broad range of training across the two centres in several vital areas of neuroscience. The two groups have recently collaborated effectively in a project on Parkinson's disease (led by Dr R Barker on the Cambridge side). The overlap in methodologies in the two groups will further facilitate a student collaborative project. Moreover, both laboratories have extensive and largely complementary expertise in appropriate animal models of cognitive function and mental illness that is tied directly to the study of human disorders

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The Control of Translesion Synthesis and Mutagensis in Vertebrates

This collaborative project will take place in the laboratories of Dr. Roger Woodgate at NIH and Dr. Julian Sale at the Laboratory of Molecular Biology in Cambridge. It will focus on understanding the in vitro activities and in vivo control of proteins involved in translesion synthesis.

The importance that translesion synthesis plays in protecting us from the deleterious consequences of exposure to environmental mutagens is most evident in humans who have defects in human DNA polymerase eta, an enzyme involved in the bypass of ultraviolet light-induced DNA lesions, and who exhibit the sunlight-sensitive and cancer-prone Xeroderma pigmentosum variant phenotype. It is also evident that humans who have been exposed to chemotherapeutic agents, are at a much higher risk of developing secondary malignancies than the general population. Thus, the use of DNA adduct forming chemotherapeutics that are used to kill rapidly growing tumor cells, may actually promote mutations that in turn leads to the development of further malignancies.

The project aims to address the mechanisms by which different DNA polymerases are selected during the bypass of a given DNA lesion and how they are controlled, so as to avoid possibly damaging unscheduled activity. It will combine in vitro studies of translesion polymerase complexes coupled with genetic and cell biological studies of these enzymes, and proteins with which they interact, in cell culture systems and mice.

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Development of the Nasal Placode and GnRH-1 Neuroendocrine Cells

The laboratories of Clare Baker, at Cambridge, and Susan Wray, at NIH, share a common interest in the development of cranial placodes - how they are induced and how multiple neuronal phenotypes differentiate from these areas. Cranial placodes are discrete regions of ectoderm in the head of the vertebrate embryo. They give rise to essential elements of the paired sense organs (eyes, ears, nose), and to different types of sensory neurons in cranial sensory ganglia. The focus of this joint project is the development of the GnRH-1 neuronal phenotype in the nasal placode. GnRH-1 (Gonadotropin-Releasing Hormone-1; also known as LHRH) neurons are neuroendocrine cells essential for reproduction. Developmentally, GnRH-1 neurons originate in the nasal placode, and thereafter migrate on olfactory axons into the brain. Once within the brain, GnRH-1 neurons become integral components of the hypothalamic-pituitary-gonadal axis. Alterations in the development of the GnRH-1 system result in reproductive dysfunctions. Multidisciplinary approaches will be used in both mouse and chick animal models to systematically manipulate the molecular and cellular biology of the developing GnRH-1/olfactory system. In particular, genes isolated from single cell libraries created from GnRH-1 neurons at different developmental stages will be characterized and perturbed to identify genes involved in their migration and differentiation. The molecular interactions that lead to the formation of GnRH-1 neurons within the nasal placode will also be analysed. These studies will lead to a better understanding of critical neurobiological issues such as phenotypic commitment and mechanisms involved in neuronal migration, processes essential for normal vertebrate development.

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Identification and characterization of novel immunomodulatory molecules produced by important disease causing parasites

Parasitic helminths, as a medically important group of pathogens, are supreme survival strategists - having evolved structures, behaviours, and mechanisms that allow for the successful continuation of life in harsh host environments such as blood, the gastrointestinal tract, and the lymphatics. Members of the genus Schistosoma are no exception. These organisms live the majority of their life (years) in the blood of their definitive hosts (vertebrate mammals) and have evolved various survival strategies allowing for the successful propagation of an infection that can cause a debilitating disease (schistosomiasis) currently affecting greater than 200 million people world-wide. In our continuing effort to understand how schistosomes actively evade host immune effector mechanisms, we have focused our search for parasite molecules that display immunomodulatory characteristics. The study of these molecules may offer insight into the understanding of host/parasite interrelationships and provide useful information towards the development of novel intervention strategies. Towards this end, we have identified several immunomodulatory molecules in the schistosome genome (via EST database analysis) and have begun to investigate the biology of two specific S. mansoni expressed transcripts, SmVen5 (orthologous to a major vespid allergen) and SmRER (orthologous to interferon inhibiting cytokine IK). These parasite transcripts share primary sequence homology to known immunomodulatory molecules and, as such, may influence the effectiveness of host generated antihelminth immune responses. This project would execute the characterisation of these schistosome transcripts and take full advantage of both host laboratories' immuno-parasitological strengths. At the University of Cambridge, schistosome immunomodulatory molecules would be characterised at the molecular and biochemical level. Developmental expression investigations, in situ localisation experiments, full-length cloning, recombinant expression studies and antisera generation against these recombinants will initially be performed. These molecular and biochemical investigations will provide a framework for cellular and immunological studies to be performed at the NIH. At the NIH, the doctoral candidate will determine how these schistosome molecules influence host immune responses by utilising in vitro cellular assays studying basophil and macrophage cell biology. The effect each molecule has on cellular biology will be monitored by a combination of techniques including FACs, ELISA, Real-Time PCR, immunohistochemical stains, and proliferation experiments. Finally, bringing all the parasitological and cell biological data together, in vivo experiments will be performed at the NIH to investigate how these schistosome molecules modulate the development of disease in murine models. Together, these studies will expand our understanding of helminth immune evasion strategies and elucidate the specific immunomodulatory activity of proteins expressed by important human parasites.

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